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EVIDENCE FOR 'BIG' AND 'LITTLE' COMPONENTS OF CIRCULATING IMMUNOREACTIVE PROLACTIN IN HUMANS

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1. Introduction

Heterogeneity of peptide hormones revealed by radioimmunoassay (RIA) has been demonstrated for a variety of peptides [1,2] including human growth hormone (hGH) [3–5]. Thus, two or three immunoreactive components of hGH, 'big-big', 'big' and 'little' hGH were shown to be present in the blood [5]. Human prolactin (hPRL) is known to be related structurally to hGH though it has different immunoreactivity. Heterogeneity of serum hPRL has been demonstrated using electrophoresis [6] and gel chromatography [7]. In order to investigate the molecular heterogeneity of hPRL we have subjected serum with high hPRL-levels from patients with prolactin producing pituitary tumors to dextran gel chromatography.

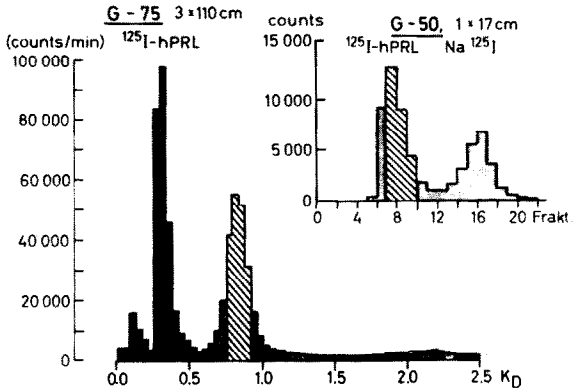

Fig. 1. Chromatography of ^{125}I-labelled VLS–hPRL on Sephadex G-50. Fractions of 1 ml were collected (upper right). The fractions 7–10 were refiltrated on Sephadex G-75 and the marked (▩) fraction of the Sephadex G-75 eluate (0.8 K_D) was used as tracer in the hPRL-radioimmunoassay.

2. Materials and methods

Human prolactin was measured by double-antibody-radioimmunoassay (RIA) using the VLS-kit [8] supplied by the National Pituitary Agency. Immunoreactive hPRL was expressed in μU using the hPRL-71/222 from the Medical Research Council, London, as reference preparation. The VLS-hPRL was labelled with ^{125}I according to Hunter and Greenwood [9] and the labelled protein purified on a Sephadex G-75 column (fig. 1).

The coefficient of variation of the hPRL-RIA from day to day was 9.1%, the lowest detectable (Bo-3 SD) hPRL-concentration 10 μU/ml. The hPRL-levels in normal females ranged from 100 to 650 μU/ml. Dextran gel filtration was performed at room temperature with Sephadex G-75 using 0.05 M phosphate buffer, pH 7.5 with 0.1% sodium azide. The column (3 × 110 cm) was saturated with albumin before each chromatography and fractions of 5 ml were collected in which hPRL was measured. VLS-hPRL-standard, pooled serum from pregnant females at term and serum from a female and a male patient with excessively high hPRL-levels were subjected to dextran gel filtration. The male patient's serum was chromatographed before and after partial hypophysectomy by transsphenoidal approach lowering the hPRL from 200 000 to 80 000 μU/ml. The serum samples were kept frozen at −18°C and thawed prior to chromatography.

3. Results

Chromatography of ^{125}I-labelled VLS-hPRL revealed three radioactive peaks (fig. 1). The first peak eluted with the void volume and was bound by the antibody but no inhibition curve with increasing concentrations of hPRL-standard could be obtained. The second peak eluting at K_D 0.4 showed no binding to the antibody whereas the third peak at K_D 0.75–0.8 bound to the antibody with satisfactory inhibition curves representing the labelled hPRL. Unlabelled immunoreactive VLS-hPRL showed marked heterogeneity (fig. 2) and immunoreactive hPRL of pregnancy serum eluted with a minor peak at K_D 0.4 and a large peak at K_D 0.75–0.8 (fig. 2).

Chromatography of serum from patients with hPRL producing pituitary tumors and very high hPRL-levels showed consistently two immunoreactive hPRL components, a 'big' hPRL with an approximate MW 44 000 representing 23–24% and a 'little' hPRL representing 76–77% of the total hPRL-immunoreactivity (fig. 3). These two components had identical

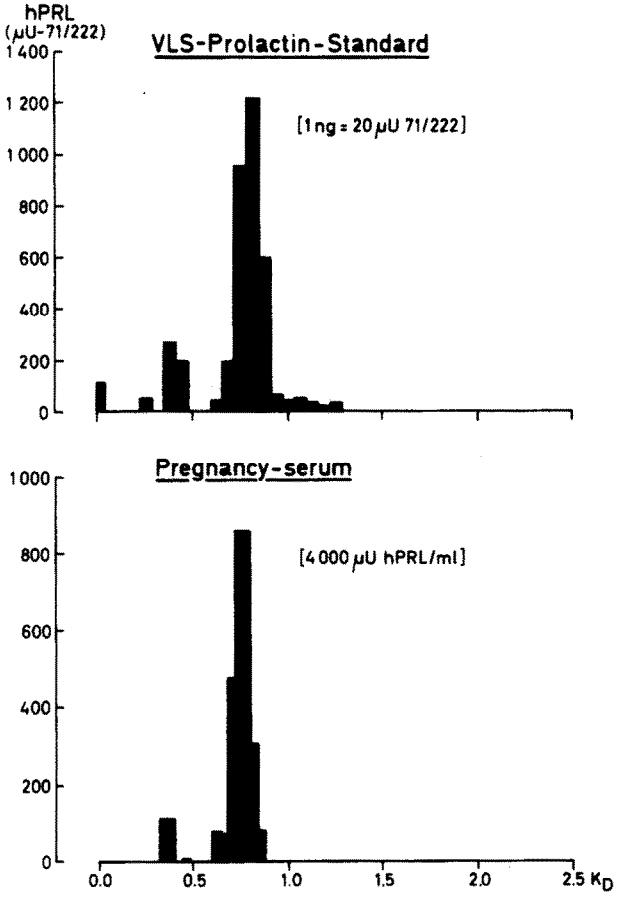

Fig. 2. Dextran gel filtration of unlabelled VLS–hPRL and pooled pregnancy serum. Two minor fractions of the VLS preparation eluted with the void volume (3%) and at K_D 0.4 (15%), the main peak (82%) at K_D 0.8 representing the regular or 'little' hPRL with mol. wt. 22 000. Pregnancy serum showed a 'big' hPRL component at K_D 0.4 (8%) and a 'little' component at K_D 0.75–08.

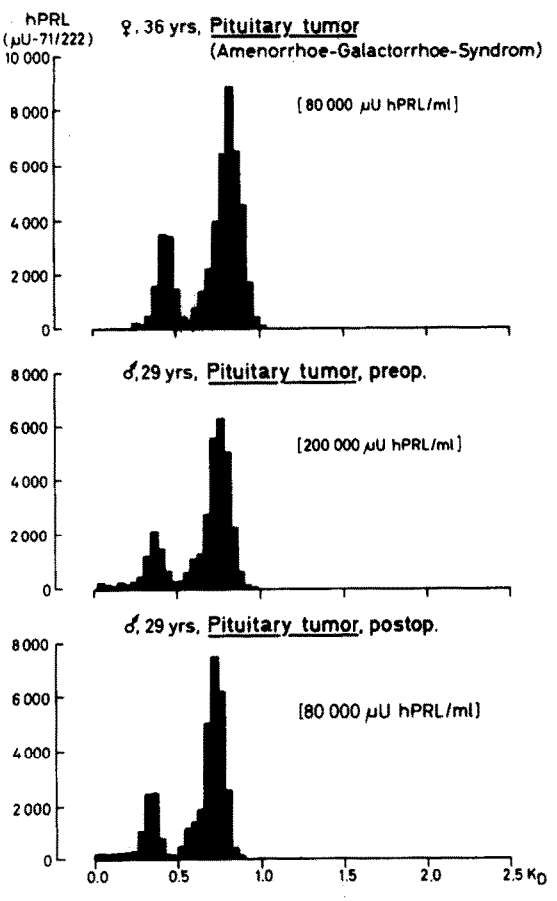

Fig. 3. Dextran gel filtration of sera with high hPRL-levels. The upper figure shows the elution pattern of the hPRL-immunoreactivity of one female patient with a hPRL-producing pituitary adenoma. A similar pattern is demonstrated in a male patient with hyperprolactinemia and pituitary tumor (middle), which did not change after lowering the hPRL-level by partial removal of the pituitary adenoma (below).

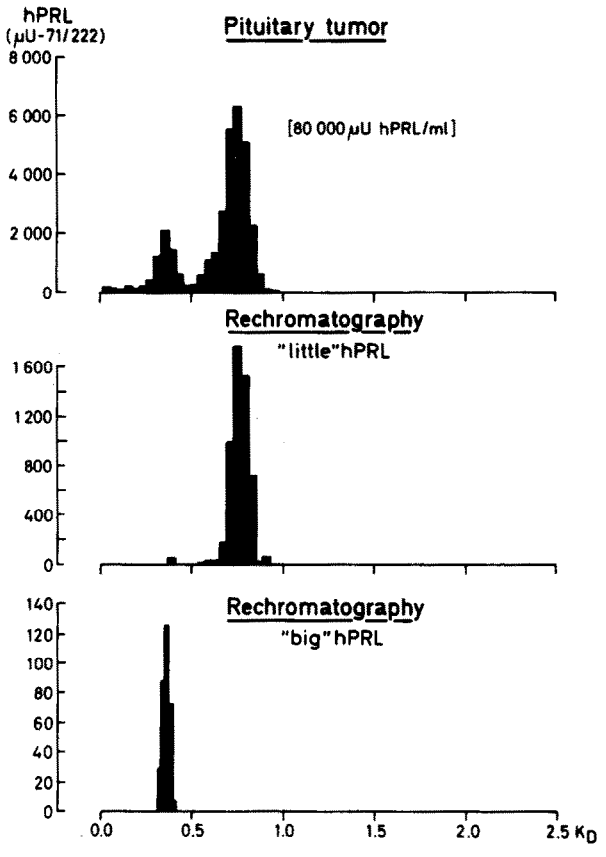

Fig. 4. Rechromatography of 'big' and 'little' hPRL-serum components. No conversion of 'big' into 'little' or vice versa could be detected.

immunoreactivity as could be demonstrated in the RIA by superimposable dilution curves.

After rechromatography 'big' hPRL eluted as 'big' and 'little' as 'little' hPRL (fig. 4) even when the samples were kept frozen prior to chromatography over several days.

4. Discussion

It has been demonstrated for most peptide hormones that the immunoreactivity can reside in more than one molecular size [1,2]. These multiple immunoreactive components have been shown to be present in their glands of origin and in the circulation. Turkington has described two forms of serum hPRL which could be separated by electrophoretic mobility into 'fast' and 'slow' moving hPRL [6]. Recently Rogol and Rosen [7] reported a 'large' hPRL as a major component in the plasma of a patient with acromegaly and elevated hPRL-levels which could be separated by Sephadex G-100 from regular hPRL. Our dextran gel studies show also two components of circulating immunoreactive hPRL. The molecular weight of 'big' hPRL according to the K_D can be estimated to be approximately 44 000 similar to the large hPRL described by Rogol and Rosen [7]. The 'big' hPRL represents 23 to 24% of total hPRL-immunoreactivity and does not correlate to the absolute hPRL concentration in the serum (fig. 3). Less 'big' hPRL was found in pregnancy serum. This might be attributed to the sensitivity of the hPRL-RIA (10 μU 71/222/ml = 0.5 ng VLS/ml) which does not allow to detect the total 'big' hPRL-fraction. 'Little' hPRL elutes at K_D 0.75 to 0.8 corresponding to mol. wt. 22 000. No conversion of 'big' into 'little' hPRL could be demonstrated by refiltration of the hPRL components, which excludes 'big' hPRL being an artifact as has been tentatively suggested [7].

These findings are similar to what has been shown for human growth hormone [5] another single chain pituitary peptide chemically related to hPRL. How these components relate to the hPRL fractions described by Turkington [6] is open. Further investigation has to clarify wether 'big' hPRL represents a hPRL-precursor, a dimer or is due to protein binding as well as its possible physiologic significance.

Acknowledgements

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